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1. Introduction

One amongst the United Nation's seventeen Sustainable Development Goals (SDG) aims to establish good health and well-being. Of the multiple aspects that contribute to the wellness, healthy and younger-looking skin plays a significant role, as is indicated by the growth of the skin-care products market (**United Nations 17 goals 2015**). Cosmeceutical is a wide market segment; especially anti-aging products are more popular and hold leading share in the market (**Cosmeceuticals market – global industry analysis 2016)**. The global cosmeceuticals market is projected to grow from $54.57 billion in 2022 to $96.23 billion by 2029, at a CAGR of 8.4% in forecast period, 2022-2029. (**Fortune Business insights 2022).**

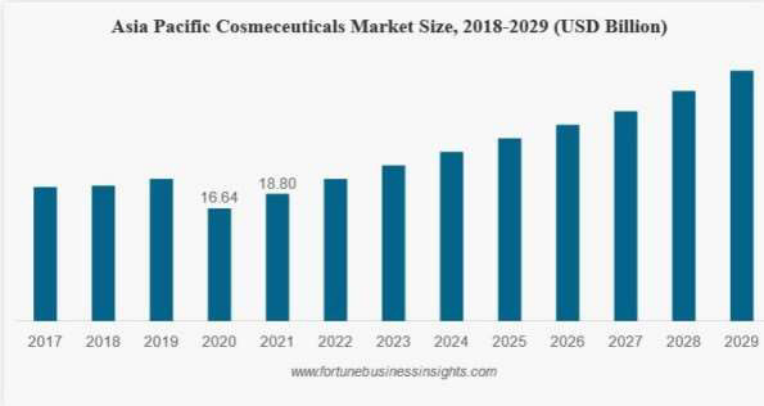

Fig1.1 Fortune Business insights 2022

1.1 Cosmeceuticals

The term "cosmeceutical" was introduced in the 1980s and has since then become a popular concept in the beauty and skincare industry. Cosmetic products that contain active ingredients that have both cosmetic and pharmaceutical properties are known as cosmeceuticals. **(Pandey, A et al., 2022)** They contain biologically active ingredients, such as vitamins and antioxidants, that penetrate into the skin to improve its health and appearance. These products target specific skin concerns, such as aging, sun damage, acne, hyperpigmentation etc.

The use of peptides in cosmeceuticals was first introduced by Pickart in 1973, who suggested GHK (glycyl-L-histidyl-L-lysine), a synthetic peptide, as a signal peptide that could boost collagen production and serve as a carrier peptide when combined with Cu (II). **(Pickart, L et al., 1973).** Since then, thanks to their versatility, a plethora of peptides of cosmeceutical interest have been developed in response to the most frequent and not fully satisfied market requests. **(Errante, F et al. 2020)** Cosmeceuticals are now preferred over cosmetics due to their ability to enhance the functioning of the skin reviving its structure. Bio actives that form a significant group of cosmeceuticals include an array of substances that alter enzymatic activity of secondary OTC (Over-the-counter) cosmeceuticals which include serums, sunscreens, lotions, and or antiperspirants.

Many of the cosmeceutical actives are derived from plant extracts. Plants contain a wide range of bioactive compounds such as polyphenols, flavonoids, terpenes, and vitamins, which possess antioxidant, anti-inflammatory, and anti-aging properties. Examples of popular plant extracts used in cosmeceuticals include green tea extract, chamomile extract, aloe vera extract, licorice extract, and rosehip extract**. (Hoang et al., 2021)** Marine sources, such as seaweed, algae, and marine plants, offer a rich array of cosmeceutical actives. These ingredients are known for their high mineral content, vitamins, and unique bioactive compounds. Marine actives can provide moisturizing, anti-aging, and skin rejuvenating effects. **(Fonseca et al.,2023)**

1.2 Rise of microalgae in cosmeceuticals

Microalgae are photosynthetic microorganisms that have been considered as a significant source of novel bioactive compounds with immense potential for diverse applications in pharmaceutical, nutraceutical, and cosmetic industries**. (Da Silva et al., 2016)** The biologically active substances present in microalgae, including pigments, polysaccharides, lipids, and proteins, possess distinct physicochemical and biological properties that make them a promising candidate for developing various cosmeceutical products. **(De Jesus et al., 2013)** The use of microalgae for various applications, including biofuels, aquaculture, food and feed production, has gained significant attention over the past few decades. Moreover, the potential of microalgae as a source of novel bioactive compounds for pharmaceutical and cosmetic industries has been extensively investigated. The unique chemical composition of microalgae and their ability to

grow rapidly and produce large amounts of biomass under different environmental conditions make them an attractive option for various industrial applications**. (Mahata c et al., 2022)** Several microalgal species have been studied for their biologically active compounds, such as carotenoids, phycobiliproteins, polysaccharides, and polyunsaturated fatty acids (PUFAs). For instance, Astaxanthin, a carotenoid present in various microalgae, has potent antioxidant and anti-inflammatory properties and is used as a dietary supplement and cosmeceutical ingredient. **(Barkia,I et al., 2019)**

Phycocyanin, a phycobiliprotein found in blue-green algae, has potent anti-inflammatory and anti-cancer properties and is used as a natural colorant and in various medicinal and cosmetic products. Moreover, fucoidan, a polysaccharide present in brown algae, has potent antiviral and anti-inflammatory properties and is used as a functional food and a cosmeceutical ingredient. **(Khavari, F et al., 2021)**

Some examples of microalgae commonly studied for their bioactive compounds are as follows:

Haematococcus: A red microalga that is known for its high astaxanthin content, a carotenoid with potent antioxidant properties. **(Oslan et al., 2021)**

Chlorella: A unicellular green microalga that is known for its high protein, chlorophyll, and nucleic acid content. It is also a rich source of carotenoids, PUFAs, and polysaccharides. **(Widyaningrum et al., 2021)**

Nannochloropsis: A unicellular green microalga that is a rich source of PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). **(Borowitzka et al., 2018)**

Spirulina: A blue-green microalga that is commonly used as a dietary supplement due to its high protein and amino acid content. It is also a rich source of phycobiliproteins, including phycocyanin and allophycocyanin**. (Wan et al., 2016)**

Dunaliella: A unicellular green microalga that is known for its high carotenoid content, particularly beta-carotene. **(Masoj dek et al., 2014)**

Chondrus crispus: - A relatively small sea alga, reaching up to a little more than 20 cm in length. is rich in fatty acid and phenols. It has the power to preserve the skin's natural protective film and it also softens the skin.

***C. tomentosum:* -** is a species of green seaweed in the family Codiaceae. Its common names include velvet horn and sponge weed. It is rich in polysaccharides. It is a hydric regulator (limits and regulates water losses) and provides a long-lasting moisturizing effect of the upper layers of the epidermis. This green alga is used in cleansing marine gel

1.3 Actives and Bioactive Peptides

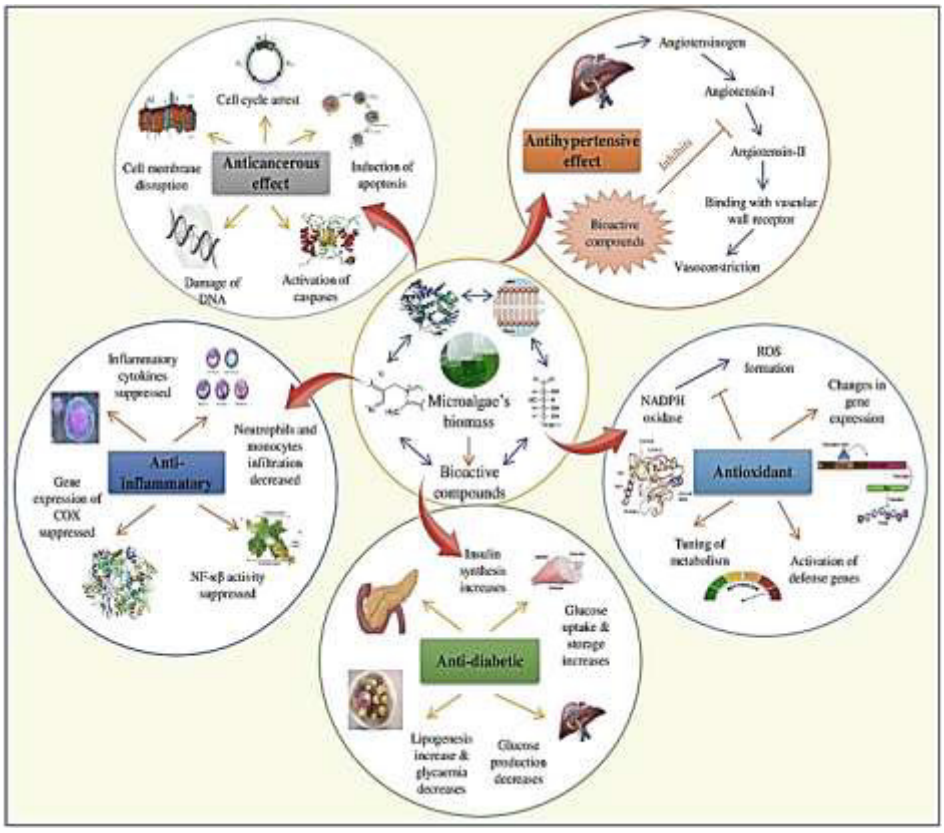

Fig1.2 The potential use of bioactive metabolites derived from microalgae in nutraceuticals and pharmaceuticals is a promising avenue for promoting health benefits (Kaur *et al*., 2023)

Microalgal actives have gained increasing attention in recent years due to their potential health benefits and diverse applications in various industries. **(Kaur et al., 2023)**

Some of the examples of Microalgal actives and hydrolastes are as follows

- **Omega-3 fatty acids**: Some species of microalgae, such *as Schizochytrium* and *Ulkenia*, are rich in omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These fatty acids are essential for human health and have been shown to have anti-inflammatory and cardiovascular benefits. **(Patel, et al., 2021)**
- **Carotenoids**: Microalgae are a rich source of carotenoids, such as astaxanthin, betacarotene, and lutein. These compounds have antioxidant properties and may offer health benefits, such as reducing the risk of chronic diseases. **(Raposo, et al.,2015)**
- **Phycobiliproteins**: These are water-soluble proteins found in some species of microalgae, such as *Spirulina*, *Chlorella* and seaweeds such as Rhodophyceae. Phycobiliproteins are known for their bright colors and have potential applications in food coloring and cosmetics. **(Matos, et al.,2019)**
- **Polysaccharides**: Microalgae produce various polysaccharides, such as alginate, carrageenan, and agar. These compounds have applications in the food, pharmaceutical, and biotechnology industries as thickening agents, gelling agents, and emulsifiers.

- **Bioactive Peptides**: Biologically active peptides are short chains of amino acids that can interact with specific receptors in the body and have a variety of physiological effects. Bioactive peptides have been obtained as is or by partial hydrolysis of protein obtained from animal, plant and microalgal source. **(Apone et al., 2019)**

Examples of biologically active peptides include:

Source	Peptide	Structure of peptide	Targeted organism
Microcystis aeruginosa TAU	Aeruginazole DA 149	Cyclic	Moxarella catharralis
Hawaii and Caribbean collection of cyanobacteria	Antillatoxin B	Lipopeptide	*Staphylococcus aureus* ATCC 29213 and *Listeria monocytogenes* HPB 2812
Tychonema sp.	Brunsvicamides A B and C	Cyclic	S. aureus
M. aeruginosa (NIES-88),	Kawaguchipeptin B	Cyclic undecapeptide	*Mycobacterium tuberculosis*
Hawaii and Caribbean collection of cyanobacteria	Laxaphycin B3	Lipopeptide	*Bacillus cereus* LSPQ 2872
Lyngbya sp 3691 SAG	Lyngbyazothrins mixture A/B	Cyclic undecapeptide	*Micrococcus flavus* SBUG 16
Synechocystis, Synechococcus and Romeria	Microcystin	Cyclic heptapeptide	*S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27,853
Lyngbya majuscula	Pitipeptolides A–F	Cyclic depsipeptide	*M. tuberculosis*
L. *majuscula*	Pitiprolamide	2,2-diMe-3-Hy-hexanoic acid and Dpv-Pro	*Bacillus cereus Mycobacterium* and *tuberculosis* H37Ra
Schizothrix sp. TAU strain IL.89-2	Schyzotrin A	Cyclic lipopeptide	*Bacillus subtilis*
Scytonema sp	Scytonemin A	Lipopeptide	*Mycobacterium* sp.
Nostoc spongieaforme var tenue	Tenuecyclamide A to D	Cyclic hexapeptide	*S. aureus B. subtilis* Bs1091-1
L. majuscula	Tiahuramide C	Cyclic depsipeptide	*Aeromonas salmonicida*
Oscillatoria sp UIC 10045	Trichormamide C	Cyclic lipopeptide	*M. tuberculosis*

Table 1.1 Various peptides derived from microalgae with antimicrobial activity (Ayswaria et al.,2023).

Source	Type of bioactive peptide (BP) preparation	Main activities
Chlorella pyrenoidosa	Isolated peptide fractions (430–1350 Da)	skin Inhibited UVB-induced MMP-1 expression in fibroblasts by suppressing AP-1, CYR61 and MCP production. Reduced UVC-induced cytotoxicity by modulating Caspase-3, FADD and ADP-ribose-PARP-1
Arthrospira platensis (Spirulina)	Enzymatic hydrolysates	Promoted hydration in skin cells by increasing AQP3, HAS3, and Filaggrin expression; counteracted osmotic stress damage by modulating Smith gene expression

Table 1.2 Examples of microalgae derived peptides used in cosmetics (Apone et al., 2019)

2. Aims of study

1. Isolation and identification of potential microalgae from marine source.
2. Optimizing growth conditions like media, light intensity etc for cultivation of marine microalgae.
3. Purification and characterization of microalgal hydrolysates including antioxidants and bioactive peptides
4. Exploring application of hydrolysates as potential cosmeceuticals.

3. Objectives of Study

1. Coastal sampling for collection of microalgae.
2. Isolation of microalgae strains from the collected samples.
3. Media optimization for obtaining maximum microalgal growth.
4. Determine the optimal light intensity and photoperiod required for the growth and photosynthetic activity of the microalgae strains.
5. To develop efficient and effective methods for induction and purification of bioactive peptides from microalgae.
6. Antioxidant profile of hydrolysates using biochemical assays like DPPH, FRAP etc.
7. To assess the toxicity and safety of microalgal bioactives and hydrolysates, using cell lines like SK-N-SH.
8. Investigate the potential anti-aging effects of the hydrolysates.

4. Study background and significance of study

There have been numerous studies focused on isolating and identifying microalgae species from marine environments. Researchers often employ various sampling techniques such as water collection, filtration, and enrichment cultures to obtain microalgal samples. The isolated microalgae are then characterized using morphological, biochemical, and molecular techniques to determine their taxonomy and potential applications. **(Kasan et al., 2020)**

The field of cosmeceuticals, which combines cosmetics and pharmaceuticals, has gained significant attention in the beauty and skincare industry. Consumers are increasingly seeking products that not only enhance their appearance but also provide additional skincare benefits. Cosmeceuticals bridge the gap between traditional cosmetics and pharmaceuticals by incorporating biologically active ingredients that can penetrate the skin and deliver specific therapeutic effects. **(Pandey et al., 2022)** One area of growing interest in the development of cosmeceuticals is the use of microalgal actives and hydrolysates. Microalgae are microscopic photosynthetic organisms that thrive in diverse environments, including marine and freshwater habitats. They have been recognized for their remarkable ability to produce a wide range of bioactive compounds with potential applications in various industries, including cosmetics and skincare. (**Stoyneva et al., 2020)**

One of the key advantages of microalgal actives is their high content of antioxidants. Antioxidants play a crucial role in protecting the skin against oxidative stress, which is caused by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms. Excessive ROS can lead to premature aging, inflammation, and damage to cellular structures. Microalgae-derived antioxidants, such as astaxanthin and fucoxanthin, have been shown to effectively scavenge free radicals and reduce oxidative damage, thereby promoting healthier and more youthful-looking skin**. (Xia et al., 2013) (Choo et al., 2020)** In addition to their antioxidant properties, microalgal actives also exhibit anti-inflammatory effects. Inflammation is a common underlying factor in various skin conditions, including acne, rosacea, and dermatitis. Active compounds derived from microalgae, such as phycobiliproteins and omega-3 fatty acids, have demonstrated anti-inflammatory activities that can help soothe and calm inflamed skin. (**Wang et al., 2017)** Moreover, microalgal actives and

hydrolysates contain bioactive peptides, which are short chains of amino acids. These peptides can offer specific benefits for the skin, such as improved moisturization, enhanced collagen synthesis, and anti-aging effects. Microalgae-derived peptides have been found to stimulate the production of collagen, a protein that provides structural support to the skin and contributes to its firmness and elasticity. By promoting collagen synthesis, these peptides can help reduce the appearance of wrinkles and fine lines, resulting in smoother and more youthful-looking skin. (**Apone et al., 2019)**

While the potential of microalgal actives and hydrolysates in cosmeceuticals is promising, further research is needed to fully understand their mechanisms of action, optimize extraction methods, assess their stability in formulations, and evaluate their safety and efficacy. Additionally, clinical studies are necessary to validate the benefits of these compounds and establish appropriate usage guidelines.

Significance of study

The study of microalgal actives and hydrolysates holds significant importance for several reasons. Firstly, these ingredients offer enhanced skincare benefits compared to conventional cosmetic products. With their antioxidant properties, they can protect the skin against oxidative stress, reducing signs of aging and promoting healthier-looking skin. Additionally, their anti-inflammatory effects make them valuable in addressing various skin conditions, providing soothing and calming effects on inflamed skin. This potential for improved skincare outcomes can greatly benefit consumers seeking effective and efficient solutions.

Another key significance of microalgal actives and hydrolysates lies in their natural and sustainable attributes. Microalgae are renewable resources that can be cultivated in a sustainable manner. By utilizing these ingredients in cosmeceuticals, it aligns with the growing demand for natural and environmentally friendly options. This aspect not only appeals to consumers who prioritize ethical and eco-conscious choices but also contributes to reducing the reliance on synthetic and potentially harmful ingredients commonly found in traditional skincare products.

Furthermore, the study of microalgal actives and hydrolysates drives scientific advancements and fosters innovation in the cosmeceutical field. By understanding the mechanisms of action and

exploring the potential of these compounds, we can expand our knowledge of skincare science. This knowledge can lead to the development of novel extraction methods, formulation techniques, and delivery systems, enabling the creation of more advanced and effective cosmeceutical products. Such advancements not only benefit the industry but also provide consumers with innovative solutions for their skincare needs, promoting continuous improvement and evolution in the field.

5. National Status

Sathya, R et al., 2021 based on survey reports, it has been concluded that microalgal peptides show potential as antioxidant, anti-cancerous, anti-hypersensitive, anti-atherosclerotic, and nutritional functional food. The research also explores innovative technologies for producing these active peptides. However, the use of algal peptides as food for human health has limitations, notably the sensitivity of these peptides to hydrolysis and protease degradation.

Ranadheer et al., 2019 reported that maximum protein production in was observed at highest sodium nitrate concentration (3X) on 8^{th} day (524 mg/g) of cultivation and low protein accumulation was found at 8^{th} day (160 mg/g) of 1X cultivation which was supported by the total chlorophyll and biomass production. Protein concentration was gradually increased up to 4^{th} day (353 mg/g) and thereafter decreased from 6^{th} (327 mg/g) and 8^{th} day (186 mg/g) of 1X cultivation, this condition may indicate that utilization of optimal nitrate concentration in the medium.

Singh et al., 2016 have reported maximum 42% antioxidant activity and cytotoxicity of *Dunaliella salina* under salinity, temperature and varying nitrogen concentrations. **Parulekar 2016,** has reported the antioxidant activity of *Chlamydomonas* sp. in terms of DPPH radical scavenging and Hydroxyl radical scavenging activity of 43%.

Chinnu et al., 2015 evaluated the antioxidant activity of an isolated beta glucan from *Chroococcus turgidus* using DPPH radical scavenging activity, Nitric oxide scavenging activity alongwith estimation of superoxide dismutase and catalase activities.

Annamalai and Nallamuthu 2014 reported the antioxidant potential of the methanolic extracts of *Chlorella vulgaris* and *Chlamydomonas reinhardtii* by hydrogen peroxide and peroxy radical scavenging assays.

Jena, J et al., 2014 Microalgae preferentially develop peptides capable of binding heavy metals and can produce organometallic complexes in the cytoplasm, thereby preventing a toxic effect.

Chaudhuri et al., 2014 have evaluated the phytochemical composition qualitatively and by HPLC as well as the antioxidant potential in terms of Hydroxyl, Nitric oxide, Superoxide and Hypochlorus acid radical scavenging activity of *Euglena tuba*.

Kumar et al., 2014 have reported the enzymatic and non-enzymatic antioxidant potential of *Chlorella vulgaris* grown in confectionary industry effluent.

Saranya et al., 2014 studied the antioxidant properties by phosphomolybdenum method, ferric reducing power, Radical scavenging potential by DPPH, Nitric oxide and Hydrogen peroxide alongwith phenolic and carotenoids content of *Chlorella salina* and *Isochrysis galbana* .

Hemalatha et al., 2013 determined the antioxidant properties of *Chlorella marina* and *Dunaliella salina* by phosphor molybdenum method, DPPH- radical scavenging assay, Hydrogen peroxide radical scavenging assay, Nitric oxide radical scavenging assay and Ferric reducing antioxidant power assay.

Nailwal and Nailwal 2013 have reported the antioxidant capacity and total phenolic content of microalgae from Kumaun Himalayan range.

Manivannan et al., 2012 have evaluated the antioxidant activity of the methanolic extract of *Chlorella marina* (Butcher, 1952) by phosphomolybdenum method, De-oxyribose radical scavenging assay and Ferric reducing antioxidant power.

6. International status

Babich, O et al., 2022 have reported Astaxanthin: a carotenoid found in microalgae such as *Haematococcus Pluvialis*, which showed to have anti-inflammatory and antioxidant properties.

Jusidin et al., 2022 have reported that the extract of Nannochloropsis oceanica, Isochrysis sp. and Thalassiosira weissflogii showed antibacterial effect against *Vibrio harveyi* at 1.0×10^5, 10^6 and 10^7 CFU mL^{-1} concentrations where *N. Oceanica* exhibited largest zone of inhibition.

Almendinger et al., 2021 have reported that Microalgae produce a variety of antioxidant secondary metabolites that could have potential health benefits. For example, *Wilmottia murravi* and *Neochloris oleobundans* showed the high content of phenolic acids (> 20 mg gallic acid eq. g^{-1}), *Phormidium* sp. has a high amount of ascorbic acid content (12–14 μmol eq. g^{-1}), and *Anisancylus obliquus* has highest content of lipid-soluble compounds.

Zhang et al., 2019 have reported that using response surface methodology (RSM), and using optimal conditions that were as follows: substrate concentration 10 mg/mL, reaction time 2.0 h, enzyme/substrate ratio (*E*/*S*) 1.9%, temperature 46.4 °C, and pH 9.2. After fractionation and separation by ultrafiltration, gel exclusion chromatography, and reversed-phase high-performance liquid chromatography, an antioxidant peptide was purified and identified as Glu-Leu-Trp-Lys-Thr-Phe by UPLC-MS/MS. The results also confirmed that Glu-Leu-Trp-Lys-Thr-Phe could significantly scavenge DPPH free radicals with an EC_{50} value of 1.514 mg mL^{-1}. It seems that the smaller molecular size and hydrophobic and/or aromatic amino acids in its sequence contributed to its antioxidant activity. Thus, *G. lemaneiformis* protein hydrolysate may be a good source of natural antioxidants.

Izadpanah et al., 2018 have reported isolation of microalgae from urban wastewater using three different light types: white, red, and blue. This process resulted in obtaining three distinct pure strains of microalgae: *Chlorella sorokiniana* IG-W-96, *Chlorella vulgaris* IG-R-96, and *Chlorella* sp. IG-B-96. The research findings revealed a significant impact of the light spectrum on the cell size of the microalgae in suspension culture.

Norzagaray-Valenzuela et al. 2017 evaluated the anti-aging potential of *Dunaliella tertiolecta*, *Tetraselmis chui* and *Nannochloropsis* sp. in terms of anti-elastase and anti-hyaluronidase activities signifying their role in preventing early aging.

Chatzikonst antinou et al., 2017 have reported the anti-tyrosinase and anti-elastase activity of *Chlorella* strains suggesting their potential in controlling skin aging and pigmentation.

Assuncao et al., 2016 have reported the antioxidant activity of 42 strains of microalgae belonging to *Cyanophyceae*, *Xanthophyceae*, *Eustigmatophyceae*, *Cryptophyceae*, *Chrysophyceae*, *Haptophyceae*, *Euglenophyceae*, Rhodophyceae and Chlorophyta by ABTS, TEAC and DPPH radical scavenging assay.

7. Study Hypotheses

The overarching goal of this study is to explore the potential of marine microalgae as a source of bioactives peptides for skincare applications. The study aims to investigate the feasibility of using the marine microalgae to produce bioactives including AMPs with significant functional properties for skin health. To achieve this, the study will be divided into several objectives.

The autotrophic culture for marine microalgae is proposed to be optimized for maximum biomass and protein synthesis by manipulating the culture conditions such as light intensity, nutrient availability, and pH conditions.

Bioactive peptides are known to be efficiently and effectively purified from microorganisms relatively fewer references from microalgae using methods such as chromatography and ultrafiltration, resulting in high-purity and high-quality bioactives.

Marine products to have unusual properties and enhanced biochemical activities. Marine Microalgal bioactive peptides may exhibit functional properties such as antioxidant, antimicrobial, and hydrating capacity.

Marine microalgal bioactives would be formulated in cosmeceuticals so as to boost their activity / effectivity and reduce their toxicity

8. Study Rationale

Coastal sampling is essential to obtain a diverse and representative collection of microalgae species. Coastal environments are known to harbour a wide range of microalgae due to their favourable conditions, including nutrient availability, sunlight exposure, and water temperature. By collecting microalgae from coastal areas, we can explore the potential for discovering new species and understanding their ecological distribution.

Isolating microalgae strains from the collected samples allows for the study of individual species in controlled laboratory conditions. By isolating specific strains, we can investigate their growth characteristics, physiological properties, and potential applications. Additionally, isolating strains helps to establish pure cultures, which are essential for conducting further experiments and characterizing the unique attributes of each strain.

Identifying the most suitable growth media for the cultivated microalgae strains is crucial for optimizing their growth and productivity. Different species may have varying nutrient requirements, and tailoring the growth media composition can provide the necessary nutrients and conditions for their optimal growth. This objective aims to determine the media compositions that promote the highest biomass production and overall health of the microalgae strains.

Light intensity and photoperiod play a significant role in the photosynthesis and growth of microalgae. Determining the optimal light conditions, including intensity and duration, can enhance the growth rate, lipid production, and other desirable characteristics of the microalgae strains. This objective aims to identify the specific light requirements that will maximize the photosynthetic activity and productivity of the cultivated microalgae.

Microalgae are a promising source of bioactive peptides with various potential applications. Developing efficient and effective methods for purifying these bioactive peptides is essential for their isolation and characterization. This objective aims to establish purification techniques that can yield high-purity bioactive peptides, which can be further studied for their biological activities and potential therapeutic applications.

Microalgal bioactive peptides have shown potential as natural antioxidants and antimicrobial agents. Evaluating the properties of these peptides, such as their antioxidant and antimicrobial

potential, is important for understanding their potential benefits and applications. This objective aims to assess the biological activities of the microalgal bioactive peptides and explore their potential as functional ingredients in various industries.

Before considering the use of microalgal bioactives and hydrolysates for practical applications, it is crucial to evaluate their safety profile. Assessing the toxicity and safety using cell lines provides an initial understanding of the potential cytotoxicity or adverse effects. This objective aims to ensure the safety of microalgal bioactives and hydrolysates, supporting their future development as functional ingredients or therapeutics.

Understanding the potential anti-aging effects of microalgal hydrolysates is valuable for the development of cosmeceuticals or skincare products. Investigating their ability to reduce wrinkles and other signs of aging can provide insights into their cosmetic.

9. Research Methodology

- **Sampling, Isolation, identification microalgae form costal habitat**

Sampling: Microalgae will be isolated from coastal area around Mumbai like Mulund, Thane creek habitat, appropriate sampling equipment and protocols will be followed to minimize contamination.

Isolation: Isolation of the single potential microalgal colonies will be carried out using techniques such as serial dilution, streaking and specific media will be used for isolation. **(Haoujar et al., 2020)**

Optimizing culture media for maximum production

Autotrophic Cultivation methods employing ASN 3, BG11, Guillard's media and is proposed to be used to acclimatize and grow the marine microalgal isolates.

Identification: Preliminary identification based on morphological characteristics using light microscopy will be performed. Molecular techniques, such as DNA sequencing will be used to determine the genetic identity of the isolated microalgae. Comparison will be done of the obtained genetic data with established microalgal databases for accurate species identification. **(Singh et al., 2015)**

Characterization: Physiological studies will be performed to assess growth rates, nutrient requirements, and environmental tolerances of the isolated microalgae. Bioactivity assays will be performed to evaluate potential applications of the isolated microalgae. This may involve assessing antioxidant activity, antimicrobial properties, or other relevant bioactive properties. Use advanced analytical techniques, such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS), to identify and quantify specific bioactive compounds produced by the microalgae. **(Sun et al., 2022)**

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- **Peptide induction and optimization.**

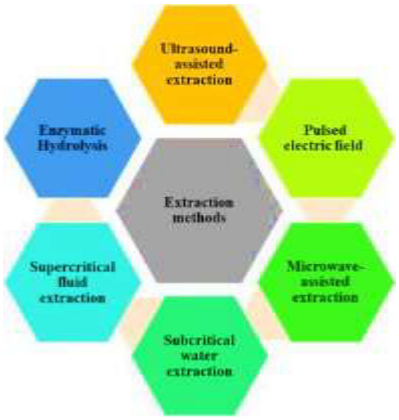

Fig 7.1 Various techniques for peptide extraction

Once optimal growth of microalgae is achieved, the proteins/peptides will be isolated with the help of various techniques such as centrifugation, enzymatic digestion, chemical extraction and/or mechanical extraction. These would then be purified with the help of filtration, chromatography and other suitable methods.

After the extraction of peptides, they would be characterized to determine their amino acid composition and properties using mass spectrometry. These would then be evaluated in vivo as well as invitro for their potential in boosting skin health.

Further optimization would be carried out to improve their delivery, effectiveness and explore their side effects if any. **(Soto-Sierra et al., 2018)**

- **Cytotoxicity**: will be assessed through cell viability assays such as MTT (3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or trypan blue exclusion test. **(Sathya et al., 2021)**

- **Anti-ageing potential**

The anti-ageing potential will be determined in terms of proteinases inhibition. Collagenase, elastase and hyaluronidase inhibition will be determined using Enzymatic assays. Also, the skin lightening potential will be determined by enzyme tyrosinase inhibition assay. **(Xia et al., 2013)**

10. Statistical plan

All experiments will be performed in quadruplet (n=4) to obtain a statistically significant data.

Results will be analysed using proper statistical techniques like ANOVA.

11. Limitations of study

- Not all isolated strains may be viable or easily cultivable, which could limit the availability of strains for further studies.
- The identified optimal growth media may be suitable only for the selected microalgae strains, and its applicability to other strains may be limited.
- The study may not extensively investigate the relationship between the structure of bioactive peptides and their specific properties, which could limit the understanding of their mode of action.

12. Expected Outcomes of the study

The isolation and identification of potential microalgal hydrolysate from a marine source aims to find actives with potential skin enhancing properties. Optimizing growth conditions, including media composition and light intensity, for the cultivation of microalgae obtained from the marine source aims to enhance their growth rate, biomass production, and overall productivity.

Purification and characterization of microalgal hydrolysates, particularly antioxidants and bioactive peptides, will provide valuable insights into their concentration, activity. Exploring the application of these hydrolysates as cosmeceuticals would enable harness their properties such as antioxidant activity, moisturization, and anti-aging effects, paving the way for potential use in skincare products to reduce wrinkles and promote healthier-looking skin.

13. Bibliography.

1. Allen, G. (Ed.). (1981). Chapter 4 Separation and purification of peptides. In *Sequencing of Proteins and Peptides* (pp. 73–133).
2. Apone, F., Barbulova, A., & Colucci, M. G. (2019). Plant and Microalgae Derived Peptides Are Advantageously Employed as Bioactive Compounds in Cosmetics. *Frontiers in Plant Science*, *10*.
3. Ayswaria, R., Vijayan, J., & Nathan, V. K. (2023). Antimicrobial peptides derived from microalgae for combating antibiotic resistance: Current status and prospects. *Cell Biochemistry and Function.*
4. Barkia, I., Saari, N., & Manning, S. R. (2019). Microalgae for High-Value Products Towards Human Health and Nutrition. *Marine drugs*, *17*(5), 304.
5. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70–76.
6. Borowitzka, M. A. (2018). Chapter 3 - Biology of Microalgae. In I. A. Levine & J. Fleurence (Eds.), *Microalgae in Health and Disease Prevention* (pp. 23–72).
7. Bendaoud, H., Bouajila, J., Rhouma, A., Savagnac, A., Romdhane, M. (2008) GC/MS analysis and antimicrobial and antioxidant activities of essential oil of *Eucalyptus radiata*. J Sci Food Agric ; 89 : 1292 – 1297.
8. Constantin, M. M., Poenaru, E., Poenaru, C., & Constantin, T. (2014). Skin Hydration Assessment through Modern Non-Invasive Bioengineering Technologies. *Maedica*, *9*(1), 33–38.
9. Choo, W. T., Teoh, M. L., Phang, S. M., Convey, P., Yap, W. H., Goh, B. H., & Beardall, J. (2020). Microalgae as Potential Anti-Inflammatory Natural Product Against Human Inflammatory Skin Diseases. *Frontiers in pharmacology*, *11*, 1086. https://doi.org/10.3389/fphar.2020.01086
10. Cosmeceuticals market – global industry analysis, size, share, growth, trends and forecasts 2016 – 2024.
11. da Silva Vaz, B., Moreira, J. B., de Morais, M. G., & Costa, J. A. V. (2016). Microalgae as a new source of bioactive compounds in food supplements. *Current Opinion in Food Science*, *7*, 73–77.
12. Dailey, M. J., & Moran, T. H. (2013). Glucagon-like peptide 1 and appetite. *Trends in endocrinology and metabolism: TEM*, *24*(2), 85–91.
13. Daskaya-Dikmen, C., Yucetepe, A., Karbancioglu-Guler, F., Daskaya, H., & Ozcelik, B. (2017). Angiotensin-I-Converting Enzyme (ACE)-Inhibitory Peptides from Plants. *Nutrients*, *9*(4), 316.
14. de Jesus Raposo, M. F., De Morais, R. M. S. C., & de Morais, A. M. M. B. (2013). Bioactivity and applications of sulphated polysaccharides from marine microalgae. *Marine drugs*, *11*(1), 233-252.
15. Errante, F., Ledwoń, P., Latajka, R., Rovero, P., & Papini, A. M. (2020). Cosmeceutical Peptides in the Framework of Sustainable Wellness Economy. *Frontiers in Chemistry*, *8*.
16. Fonseca, S., Amaral, M. N., Reis, C. P., & Custódio, L. (2023). Marine Natural Products as Innovative Cosmetic Ingredients. *Marine drugs*, *21*(3), 170.

17. Froehlich J. C. (1997). Opioid peptides. *Alcohol health and research world*, *21*(2), 132–136.
18. GVR Report cover Anti-Aging Products Market Size, Share & Trends Report Anti-Aging Products Market Size, Share & Trends Analysis Report by Product (Facial Cream & Lotion, Eye Cream & Lotion), By Distribution Channel (Hypermarket & Supermarket, Specialty Store), And Segment Forecasts, 2021 – 2028.
19. Guzmán, F., Wong, G., Román, T., Cárdenas, C., Alvárez, C., Schmitt, P., Albericio, F., & Rojas, V. (2019). Identification of Antimicrobial Peptides from the Microalgae *Tetraselmis suecica* (Kylin) Butcher and Bactericidal Activity Improvement. *Marine drugs*, *17*(8), 453.
20. Hoang, H. T., Moon, J.-Y., & Lee, Y.-C. (2021). Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives. *Cosmetics*, *8*(4), 106. MDPI AG. Retrieved from
21. Haoujar, I., Cacciola, F., Manchado, M., Abrini, J., Haoujar, M., Chebbaki, K., Oteri, M., Rigano, F., Mangraviti, D., Mondello, L., Essafi, A., Chairi, H., & Skali Senhaji, N. (2020). Isolation of Microalgae from Mediterranean Seawater and Production of Lipids in the Cultivated Species. *Foods (Basel, Switzerland)*, *9*(11), 1601.
22. https://www.un.org/en/exhibits/page/sdgs-17-goals-transform-world (2015).
23. Izadpanah, M., Gheshlaghi, R., Mahdavi, M. A., & Elkamel, A. (2018). Effect of light spectrum on isolation of microalgae from urban wastewater and growth characteristics of subsequent cultivation of the isolated species. *Algal Research*, *29*, 154–158.
24. Jayshree, A., Jayshree, S., Thangaraju, N. (2016) *Chlorella vulgaris* and *Chlamydomonas reinhardtii:* Effective antioxidant, antibacterial and anticancer mediators. Indian J Pharm Sci; 78 (5): 575 – 581.
25. Kasan, N., Shahirah, F., Haris, N., Zakaria, M., Mohamed, N., Rasdi, N., Jusoh, M. (03 2020). Isolation of freshwater and marine indigenous microalgae species from Terengganu water bodies for potential uses as live feeds in aquaculture industry. *International Aquatic Research*, 74–83.
26. Kaur, M., Bhatia, S., Gupta, U., Decker, E., Tak, Y., Bali, M.,Bala, S. (2023). Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: inspiring therapy for health benefits. *Phytochemistry Reviews*.
27. Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, *17*(1), 36.
28. Khavari, F., Saidijam, M., Taheri, M., & Nouri, F. (2021). Microalgae: therapeutic potentials and applications. *Molecular biology reports*, *48*(5), 4757–4765.
29. Kumar, N., Sood, D., Tomar, R., & Chandra, R. (2019). Antimicrobial Peptide Designing and Optimization Employing Large-Scale Flexibility Analysis of Protein-Peptide Fragments. *ACS Omega*, *4*(25), 21370–21380.
30. Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D. H., & He, Q. (2019). The antimicrobial peptides and their potential clinical applications. *American journal of translational research*, *11*(7), 3919–3931.
31. Li, Y., Lammi, C., Boschin, G., Arnoldi, A., & Aiello, G. (2019). Recent Advances in Microalgae Peptides: Cardiovascular Health Benefits and Analysis. *Journal of Agricultural and Food Chemistry*, *67*(43), 11825–11838.
32. Li, Y., Zhou, W., Hu, B., Min, M., Chen, P., & Ruan, R. R. (2011). Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains

screening and significance evaluation of environmental factors. *Bioresource technology*, *102*(23), 10861–10867.
33. Mahata, C., Das, P., Khan, S., Thaher, M. I. A., Abdul Quadir, M., Annamalai, S. N., & Al Jabri, H. (2022). The Potential of Marine Microalgae for the Production of Food, Feed, and Fuel (3F). *Fermentation*, *8*(7), 316.
34. Masojídek, J., & Torzillo, G. (2008). Mass Cultivation of Freshwater Microalgae. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 2226–2235).
35. Matos, Â. P. (2019). Chapter 3 - Microalgae as a Potential Source of Proteins. In C. M. Galanakis (Ed.), *Proteins: Sustainable Source, Processing and Applications* (pp. 63–96).
36. Martínez-Ruiz, M., Martínez-González, C. A., Kim, D. H., Santiesteban-Romero, B., Reyes-Pardo, H., Villaseñor-Zepeda, K. R., Meléndez-Sánchez, E. R., Ramírez-Gamboa, D., Díaz-Zamorano, A. L., Sosa-Hernández, J. E., Coronado-Apodaca, K. G., Gámez-Méndez, A. M., Iqbal, H. M. N., & Parra-Saldivar, R. (2022). Microalgae Bioactive Compounds to Topical Applications Products-A Review. *Molecules (Basel, Switzerland)*, *27*(11), 3512.
37. Oslan, S. N. H., Tan, J. S., Oslan, S. N., Matanjun, P., Mokhtar, R. A. M., Shapawi, R., & Huda, N. (2021). *Haematococcus pluvialis* as a Potential Source of Astaxanthin with Diverse Applications in Industrial Sectors: Current Research and Future Directions. *Molecules (Basel, Switzerland)*, *26*(21), 6470.
38. Pandey, A., Jatana, G. K., & Sonthalia, S. (2022). Cosmeceuticals. In *StatPearls*. StatPearls Publishing.
39. Parulekar, G.R. (2016) Antioxidant activity of freshwater microalga *Chlamydomonas sp*. International Journal Pure Applied Bioscience; 4 (5): 55 – 58.
40. Patel, A., Karageorgou, D., Katapodis, P., Sharma, A., Rova, U., Christakopoulos, P., & Matsakas, L. (2021). Bioprospecting of thraustochytrids for omega-3 fatty acids: A sustainable approach to reduce dependency on animal sources. *Trends in Food Science & Technology*, *115*, 433–444
41. Pickart, L., Thayer, L., & Thaler, M. M. (1973). A synthetic tripeptide which increases survival of normal liver cells, and stimulates growth in hepatoma cells. *Biochemical and biophysical research communications*, *54*(2), 562–566.
42. Raposo, M. F., de Morais, A. M., & de Morais, R. M. (2015). Carotenoids from Marine Microalgae: A Valuable Natural Source for the Prevention of Chronic Diseases. *Marine drugs*, *13*(8), 5128–5155.
43. Ranadheer P, Kona R, Sreeharsha RV, Venkata MS (2019) Non-lethal nitrate supplementation enhances photosystem II efficiency in mixotrophic microalgae towards the synthesis of proteins and lipids. Bioresour Technol 283:373–377
44. Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety*, *1*(1), 29–46.
45. Stoyneva-Gärtner, M., Uzunov, B., & Gärtner, G. (2020). Enigmatic Microalgae from Aeroterrestrial and Extreme Habitats in Cosmetics: The Potential of the Untapped Natural Sources. *Cosmetics*, *7*(2), 27.
46. Sathya, R., MubarakAli, D., MohamedSaalis, J., & Kim, J.-W. (2021). A Systemic Review on Microalgal Peptides: Bioprocess and Sustainable Applications. *Sustainability*, *13*(6), 3262.
47. Singh, P., Baranwal, M., Reddy, S.M. (2016) Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress. Pharm Biol ; 54 (10) : 2269 – 2275.

48. Singh, P., Gupta, S. K., Guldhe, A., Rawat, I., & Bux, F. (2015). Chapter 4 - Microalgae Isolation and Basic Culturing Techniques. In S.-K. Kim (Ed.), *Handbook of Marine Microalgae* (pp. 43–54). doi:10.1016/B978-0-12-800776-1.00004-2
49. Soto-Sierra, L., Stoykova, P., & Nikolov, Z. L. (2018). Extraction and fractionation of microalgae-based protein products. *Algal Research*, *36*, 175–192.
50. Sun, K.-L., Gao, M., Wang, Y.-Z., Li, X.-R., Wang, P., & Wang, B. (2022). Antioxidant Peptides from Protein Hydrolysate of Marine Red Algae Eucheuma cottonii: Preparation, Identification, and Cytoprotective Mechanisms on H2O2 Oxidative Damaged HUVECs. *Frontiers in Microbiology*, *13*.
51. Wan, D., Wu, Q., & Kuča, K. (2016). Chapter 42 - Spirulina. In R. C. Gupta (Ed.), *Nutraceuticals* (pp. 569–583)
52. Widyaningrum, D., & Prianto, A. D. (2021). Chlorella as a Source of Functional Food Ingredients: Short review. *IOP Conference Series: Earth and Environmental Science*, *794*(1), 012148.
53. Wang, H.-M. D., Li, X.-C., Lee, D.-J., & Chang, J.-S. (2017). Potential biomedical applications of marine algae. *Bioresource Technology*, *244*, 1407–1415.
54. Xia, S., Wang, K., Wan, L., Li, A., Hu, Q., & Zhang, C. (2013). Production, Characterization, and Antioxidant Activity of Fucoxanthin from the Marine Diatom Odontella aurita. *Marine Drugs*, *11*(7), 2667–2681.
55. Zhan, J., Rong, J., & Wang, Q. (2016). Mixotrophic Cultivation, a Preferable Microalgae Cultivation Mode for Biomass/bioenergy Production, and Bioremediation, Advances and Prospect. *International Journal of Hydrogen Energy*, *42*.
56. Zhang, X., Cao, D., Sun, X., Sun, S., & Xu, N. (2019). Preparation and identification of antioxidant peptides from protein hydrolysate of marine alga *Gracilariopsis lemaneiformis*. *Journal of Applied Phycology*, *31*(4), 2585–2596.